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MOLECULAR CLONING OF THE HUMAN *ERCC4* DNA REPAIR GENE, THE CANDIDATE *XPF* GENE AND HOMOLOG OF *MEI-9* AND *RAD1*. K.W. Brookman, J.E. Lamerdin*, A.V. Carrano, and L.H. Thompson, Biology and Biotechnology Research Program, Lawrence Livermore National Laboratory, P. O. Box 808, Livermore, CA 94551.

ERCC4 is a one of a set of genes functioning in the nucleotide excision repair pathway, which is represented by 11 complementation groups of rodent cell mutants and 7 complementation groups in the human disorder xeroderma pigmentosum. Probes from a genomic clone of *ERCC4* that partially restores UV resistance to CHO cell mutant UV41 (rodent group 4) (1) were used to screen a human cDNA library prepared in vector pEBS7 provided by Randy Legerski. Five isolates were acquired, and another clone was purified from the pcD2 SV40 expression library (Okayama) using a cDNA fragment as probe. Inserts, ranging from 0.7 to 2.1 kb, were sequenced either partially or entirely. Comparison against the genomic sequence (cosmid pER4-6) revealed coding sequences interrupted by introns in all library clones. Gene-specific primers coupled with anchored 5' and 3' oligonucleotides were used to obtain the missing cDNA fragments by reverse transcriptase/PCR. Northern blot analysis of *ERCC4* in HeLa and mouse mRNAs showed messages of 6.7 kb and 3.8 kb, which agrees with the 3857 bp transcript predicted by RT/PCR. To reconstitute the cDNA, library and RT/PCR fragments were joined with cosmid-derived PCR fragments in *neo*-containing expression vector pcDNA3. The full-length cDNA (clones cER4-40->44) and a version with a truncated 3' UTR (clones cER4-30->34), were transfected into UV41 to test for correction. Transformants were generated by all constructs following exposure to either cross-linking agent mitomycin C (20 nM) or 260 nm UV light (5 J/sq meter) and 1.7 mg/ml Geneticin. The transfer frequency of UV^r plus $Geneticin^r$ was ~ 1 x 10^{-4} for 5 μg DNA, and the degree of correction among these clones ranged up to ~75%. Alignments of the predicted 916-amino-acid open reading frame show homology with *S. cerevisiae* RAD1, *S. pombe* rad16, and Drosophila meiotic recombination protein MEI-9 (54%, 58%, and 61% similarity, respectively). The *ERCC4* minigene is currently being assayed for ability to correct UV sensitivity in XP complementation group F. (Work done under the auspices of the U.S. DOE by LLNL under contract No. W-7405-ENG-48.)

1. Thompson, L.H, Brookman, K.W., Weber, C.A., Salazar, E.P., Reardon, J.T., Sancar, A., Deng, Z., and Siciliano, M.J. (1994). Molecular cloning of the human nucleotide-excision-repair gene *ERCC4*. *Proc. Natl. Acad. Sci. USA* **91**,6855-6859.